Pharmacokinetic and pharmacological study of SAK3, a novel therapeutic drug candidate for Alzheimer's Disease（アルツハイマー病治療薬候補SAK3の作用機序と脳内動態に関する研究）

博士論文内容要旨

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（アルツハイマー病治療薬候補 SAK3 の作用機序と脳内動態に関する研究）

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【Introduction】

Alzheimer's disease (AD) is the most common form of dementia in the world characterized by the neuropathological hallmarks consisting of an accumulation of extracellular amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles (NFT), followed by neuronal loss. AD incidence increases with population aging and causes a heavy burden for families and society. Currently, cholinesterase inhibitors and the N-methyl-D-aspartate receptor antagonist memantine are the only available options in the clinical, but these drugs have only symptomatic relief and do not affect disease progression. Therefore, several therapeutics targeting other molecule, such as Aβ and tau, are continued to be developed. In the present study, we developed a new therapeutic candidate for AD SAK3, a strong TVGCC enhancer and revealed its pharmacokinetics and therapeutic effects against AD-related pathology using new AD model mice, $App^{NL-F/NL-F}$ (NL-F) and $App^{NL-G-F/NL-G-F}$ (NL-G-F) knock-in mice.

【Materials and methods】

We investigated the pharmacokinetics of SAK3¬-d10 and assessed whether SAK3 administration ameliorated AD-related pathology as followed.

(i) Mice were orally administered with SAK3¬-d10 (0.5 mg/kg) followed by the plasma

and brain were collected at some time points. Pharmacokinetics of SAK3¬-d10 were revealed by determining the concentration using UPLC-MS/MS systems. Moreover, we investigated whether AD pathology influences the pharmacokinetics of SAK3¬-d10 using 8- and 15-month-old NL-G-F mice.

(ii) NL-F mice were orally administered with SAK3 (0.5 mg/kg) for 3 months. After administration, AD-related pathology including cognitive decline and Aβ deposition were analyzed by behavioral tests, ELISA and immunohistochemistry. Additionally, we conducted microarray and RT-qPCR analyses to investigate the change of mRNA expression by SAK3 administration.

(iii) NL-G-F mice were acutely administered with SAK3 (0.5 mg/kg) and subjected the behavioral tests to assess the cognitive function. Additionally, microdialysis study was conducted to investigate the release of Acetylcholine (ACh) in NL-G-F mice.

(iv) NL-G-F mice were orally administered with SAK3 (0.5 mg/kg) for 3 months. After administration, the changes in proteasome activity were assessed. Moreover, CaMKII/Rpt6 signaling was investigated by western blot analysis. Furthermore, spine abnormalities were analyzed using lucifer yellow injection method. Finally, cognitive function was assessed by behavioral tasks.

【Results】

(i) After administration, SAK3-d10 was rapidly absorbed and reached its peak concentration (Cmax: 17.8 ± 1.8 nM) at about 0.65 ± 0.15 h (t_{max}) and subsequently disappeared quickly from the blood. In the brain, SAK3-d_{10} rapidly reached to C_{max} (454.5 ± 66.4 pM) at approximately 0.70 ± 0.12 h. Unexpectedly, brain concentration of SAK3-d_{10} was not changed in NL-G-F mice, even at 15-month-old.

(ii) Chronic SAK3 administration significantly improved the decreased cognitive function in NL-F mice. Additionally, Aβ deposition was markedly decreased by chronic SAK3 treatment. Microarray and RT-qPCR analyses revealed that SAK3 administration ameliorated the declined mRNA expression of serum- and glucocorticoid-induced protein kinase 1 (SGK1) in NL-F mice. On the other hand, mRNA expression involved in APP metabolism were not changed.

(iii) Acute SAK3 administration significantly rescued cognitive impairment found in NL-G-F mice. In microdialysis study, SAK3 treatment increased the decreased the release of ACh in the hippocampus of NL-G-F mice to the basal levels of wild-type mice.

(iv) SAK3 administration significantly increased proteasome activity decreased in NL-G-F mice brain. The decrease autophosphorylated CaMKII and phosphorylation of Rpt6 were significantly restored by SAK3 administration. Spine abnormalities including decreased spine densities and increased immature spines were ameliorated by SAK3 administration. Cognitive impairments in NL-G-F mice were markedly improved by SAK3 treatment.

【Conclusion】

In this study, we developed a new AD drug candidate SAK3, a strong TVGCC enhancer and revealed its pharmacokinetics and therapeutic effects against AD-related pathology using new AD model mice, NL-F and NL-G-F mice.

Firstly, we observed that SAK3 acute and chronic treatment rescued cognitive decline in NL-F and NL-G-F mice. Moreover, chronic SAK3 administration significantly decreased Aβ deposition, one of the hallmarks of AD. We found one possible gene, SGK1 involved in the effects by SAK3 treatment using microarray and RT-qPCR analyses,

though detailed mechanism remained unclear. In acute administration study, we clarified that SAK3 could increase the declined Ach release in the hippocampus of NL-G-F mice, contributing to the cognitive improvement.

Next, we revealed one possible mechanism involved in the therapeutic effects of SAK3 against AD pathology. Chronic SAK3 administration improved the decreased proteasome activity in NL-G-F mice via activation of CaMKII/Rpt6 signaling. Increased proteasome activity accounts for the inhibition of Aβ accumulation and the improvement of spine abnormalities, contributing cognitive improvement.

Taken together, we propose that SAK3 may be a new attractive drug candidate for AD having a new mechanism of action.

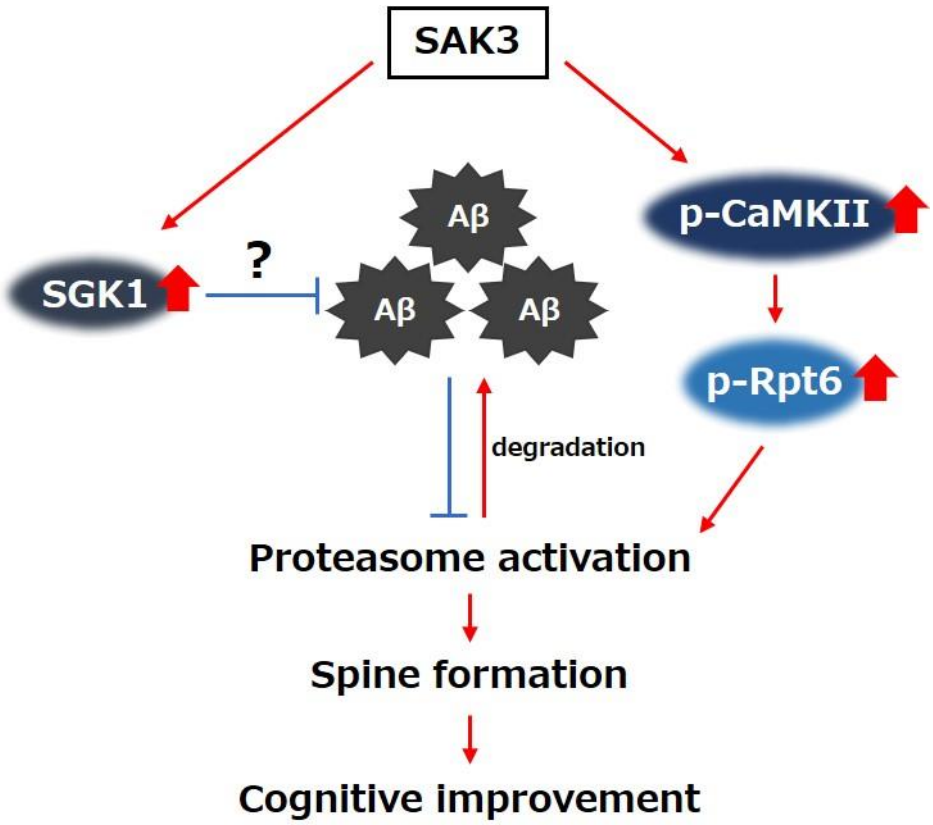

Fig.1 Schematic depicting of the signaling pathway involved in the therapeutic effects by SAK3 treatment against AD pathology.

別紙様式（Ａ４版）

論文審査結果の要旨

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論 文 題 目： Pharmacokinetic and pharmacological study of SAK3, a novel therapeutic drug candidate for Alzheimer’s Disease（アルツハイマー病治療薬候補 SAK3 の作用機序と脳内動態に関する研究）

アルツハイマー病（AD）はアミロイドベータ（Aβ）沈着と神経原線維変化を病理学的特徴とする進行性の神経変性疾患である。現在、AD の治療にはコリンエステラーゼ阻害剤であるドネペジル、リバスチグミンと NMDA 受容体拮抗薬であるメマンチンが使用されているが、それらは対症療法であり、AD の進行を抑止する効果はない。SAK3 は T 型カルシウムチャンネルを活性化して、認知機能を改善する低分子であるが、Aβ 沈着への効果、そのメカニズムについては不明である。さらに、SAK3 の脳移行性について不明である。本研究では SAK3 の脳移行性を確認して、Aβ 沈着の抑制効果と認知機能改善効果のメカニズムについて検討し、次の結果を得た。1）UPLC-MS/MS を用いた解析で、野生型マウスの脳内移行性を確認した。2）野生型マウスと AD マウス（NL-G-L）マウスの海馬において、アセチルコリン遊離を高める。既存薬のドネペジルにはこの効果は見られない。3）SAK3 の慢性経口投与は CaMKII/Rpt6 シグナルを活性化してプロテアソーム活性を上昇させ、アミロイドプラークの分解を促進することが示唆された。4）プロテアソーム活性化は AD マウスで障害されているスパイン形成を改善する効果にも関与すると考えられる。

以上、本研究では新規 T 型カルシウムチャネル活性化薬が脳内に移行すること、アセチルコリン遊離作用を有することを明らかにした。次に、SAK3 は細胞内カルシウムを上昇させることにより、CaMKII を活性化し、続いてプロテアソームのサブユニットである Rpt6 をリン酸化して、プロテアソーム活性を上昇させることを明らかにした。プロテアソーム活性上昇は、Aβ 沈着抑制に加えて、スパイン構造を改善することで AD マウスの認知機能を改善することが示唆される。現在、Aβ 沈着抑制を抑制する AD 治療薬はない。SAK3 はプロテアソームを介した新規メカニズムを明らかにして、臨床開発に進む可能性を示した点で大いに評価できる。

よって，本論文は博士（薬科学）の学位論文として合格と認める。